

# Correlation of HPV antigen and type of genital warts with atypia

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## Abstract

**Five clinical types of genital warts were identified, studied histopathologically and stained for human papilloma virus (HPV) antigen. HPV antigen was found in all degrees of koilocytosis and it was present more often in the hyperplastic warts than in other types of genital warts. However, as reported earlier, the presence of HPV antigen did not decrease proportionately as the degree of atypia increased.**

## Introduction

Human papilloma viruses (HPVs) have been known to produce neoplasias and hyperplasia in man, but certain types (HPV 16, 18, 31, 33 and 35) have a greater oncogenic potential.<sup>1</sup> It is important to recognise these potentially oncogenic virus induced warts.<sup>2-4</sup> Histopathology has been used for this, and a few unsuccessful attempts were made to look for virus type specific cytopathic effects and correlate these with routine histology.<sup>5,6</sup> Immunologic reagents can be used to detect genus specific viral antigens in tissues.<sup>7</sup>

HPVs produce various clinical types of lesions on the genitalia, with subtle histopathological changes, including koilocytosis. Anti-HPV sera can enlarge the range of cells acceptable as koilocytes.<sup>8</sup> The degree of koilocytosis and atypia varies with the type of wart.<sup>6,9</sup> The present study was intended to find out the relationship between koilocytosis, cellular dysplasia and viral antigen expression in various types of genital warts.

## Material and methods

A total of 195 patients with genital warts were included which accounted for 21% of the total patients with sexually transmitted diseases. Out of the 195 patients, 50 were selected randomly to

represent every clinical type of wart. All the warts were classified purely on a clinical basis by at least two observers independently. Biopsy sections taken from the genital warts were stained with haematoxylin and eosin. Sections were studied for human papilloma virus antigen using immunohistochemical methods employing a peroxidase-antiperoxidase (PAP) technique.<sup>10</sup> Genus specific antipapilloma virus antisera (Dakopatts, Denmark) was used for the detection of HPV antigen.

The intensity of staining reaction for viral antigen was graded from 0 to + + + indicative of negative to strong positive.

Histopathology sections were specifically screened for koilocytosis and dysplasia in addition to other epidermal and dermal changes.

## Results

The warts were clinically classified into five types:

1. Hyperplastic or classical: exophytic, proliferative warts, flesh coloured or greyish located mostly in the moist areas.
2. Sessile: seen as discrete small, skin coloured, hyperpigmented or greyish white lesions, dry or moist, usually less than 5 mm in size having granular rough surface, mostly situated on dry areas but in moist location as well.
3. Verruca vulgaris like: large dry hyperkeratotic lesions with rough verrucous surface and of grey to dark grey/brown colour, present on perineum, pubis, scrotum, etc.
4. Pigmented papules: discrete papules with shiny smooth surface, always pigmented lightly or darkly, could be present anywhere, mostly in combination with other types of warts, rarely alone.
5. Giant condylomata acuminata (Buschke-Lowenstein tumour) infiltrated plaque or noduloplaque, large, gradually progressive and locally destructive (repeated biopsies failed to show any evidence of malignancy).

Lesions with any inter-observer variation were not included. Out of the 50 specimens available for study, there were 23 of classical warts, 13 of sessile warts, three verruca vulgaris like, nine were pigmented papules and only two were giant condylomas.

*Histopathology:* All the warts had certain common

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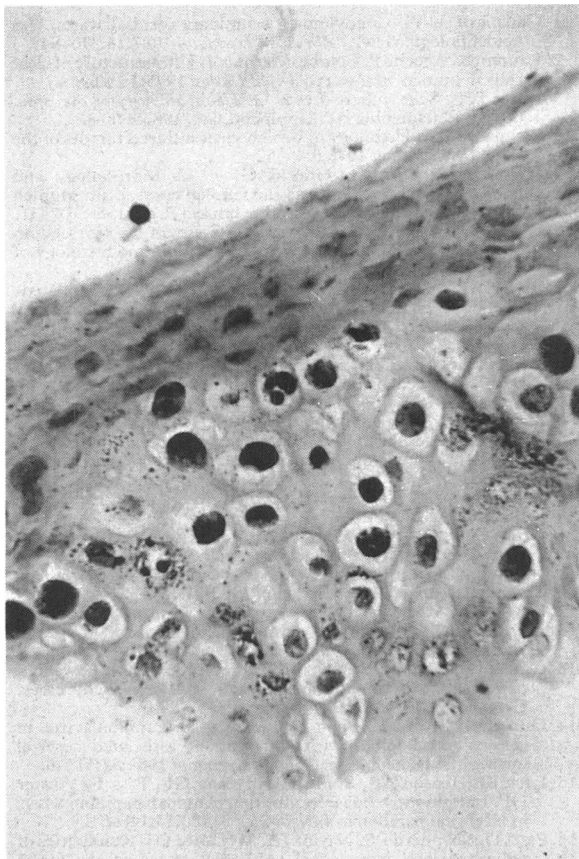


Figure Typical PAP reaction in a genital wart showing presence of HPV antigen in the nuclei of koilocytotic cells with marked anisonucleosis.

histopathological characteristics viz. hyper-keratosis, parakeratosis, focal or diffuse granulositis, acanthosis, elongation and widening of rete ridges, papillomatosis, dilatation and proliferation of dermal capillaries and mild chronic inflammatory infiltrates. The degree of hyperkeratosis varied in various types of warts, being more in hyperplastic type and less in sessile type. Koilocytosis, dyskeratosis and nuclear atypia were seen to a varying degree. Koilocytosis was graded as 0 to 4 as described earlier,<sup>11</sup> and cellular atypia from types 1 to 3. An inverse relationship between koilocytosis and atypia was observed as koilocytosis was absent when severe atypia was present.

On PAP stain (fig), viral antigen was present only in the nuclei of vacuolated or flattened cells of the superficial epithelium and found only in some of the cells displaying viral cytopathological changes. None of the cells without frank koilocytosis was positive. Seventeen of 25 specimens with type 1 koilocytosis were positive for viral antigen. Four out of the nine

with type 3 changes were positive, all were positive with type 4 changes. None of the specimens showing type 2 koilocytosis was positive. Four out of seven with mixed pattern of koilocytosis were also positive. There was evidently no definite correlation between the degree of koilocytosis and percentage positivity for viral antigens.

Papilloma virus was detected most frequently in the classical type of hyperplastic warts (19/23) followed by sessile warts (6/13). One out of three cases of verruca vulgaris type, two of the nine pigmented papules and none from giant condylomata showed a positive reaction. The maximum amount (+ + +) of the antigen was detected in the hyperplastic warts, followed by sessile warts. Moderate reaction was observed in verruca vulgaris type of warts and pigmented papules.

Viral antigen positivity did not diminish significantly with the increase in the degree of cellular atypia. It was found in 52.9% of the specimens with no atypia, in 83.3% of the specimens showing type 1 changes, in 37.5% with type 2 atypia and in 40% of the specimens showing severe (type 3) atypia.

### Discussion

This study reaffirms the presence of HPV antigen in the nuclei of the cells in the superficial and intermediate layers showing typical vacuolation (koilocytotic change). It has been earlier documented that the presence of HPV antigen decreased markedly as features of dysplasia (intraepithelial neoplasia) became apparent, because the virus replicates in the quiescent cells in the upper layers compared with cells with greater atypia or increased mitotic activity.<sup>8,11</sup> However, for unknown reasons the expression of virus has been found to be positive more often in rapidly proliferating hyperplastic type of lesions compared with the flat type of warts.<sup>12</sup> We also found the viral antigen more frequently in the hyperplastic warts followed by sessile warts and minimal in keratinised verruca vulgaris type (less actively growing) lesions.

The low rate of antigen detection reported in pigmented papules is probably because of incomplete virus particle expression or that the lesion contains less number of viral particles.<sup>13</sup> Histopathologically also, pigmented papules do not seem to be rapidly proliferating.

Our findings, however, do not support the observation of significant reduction in viral antigen positivity in grade 2 and 3 dysplastic cells. Correlation between viral antigen positivity rate and dysplasia was not significant in our study.

The presence of viral antigen detected by the immunologic test employed is indicative of productive viral infection. The absence of viral antigen positivity even in the presence of actively replicating virus in more dysplastic cells<sup>12,14</sup> may be due to

inability of the transformed cell to synthesise viral particles or due to the periodic expression of the mature virus. In patients with HPV induced laryngeal papilloma, the detection rate for HPV by immunoperoxidase analysis increased from 48% in a single biopsy to 90% with multiple biopsies.<sup>15</sup> Figures for antigen positivity in condylomas have varied widely but has been lower than the expected rate of near 100%,<sup>9 11 12</sup> but should improve if more specimens are studied at extended periods.

Widely variable HPV positivity rates may not solely be due to different techniques but also because of the variable host cell responses and unpredictable viral characters, namely periodicity of viral expression and maturity of virus. The types of reagents employed are also important.<sup>13</sup> It has been highlighted by Patel *et al*<sup>16</sup> that while polyclonal antisera extensively cross react with different HPV types, monoclonal antibodies are far more specific. However, no attempt was made to type the HPV in this study. Lastly oncogenic viruses are less likely to be detected by immunohistochemistry<sup>17</sup> and DNA hybridisation is a more definite indication of infection.

It can be concluded that there is no correlation between the clinical type, histopathology and immunohistological observations in HPV induced lesions. Contrary to the earlier reports, the dysplastic cells also gave positive staining for HPV, and increasing degree of dysplasia does not always mean reduced percentage of HPV staining.

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- 1 Chuang Tsu-Yi. Condylomata acuminata (genital warts). An epidemiologic view. *J Am Acad Dermatol* 1987;16:376-84.
- 2 Laurent R, Agoche P, Coume-Marquet J. Ultrastructure of clear cells in human viral warts. *J Cell Pathol* 1975;2:140-8.
- 3 Lever WF, Schaumburg-Lever G. *Histopathology of the Skin*. 6th ed. Philadelphia: JB Lippincott Co., 1983:376-8.
- 4 Steigleder GK. Histology of benign virus induced tumors of the skin. *J Cell Pathol* 1978;5:45-52.
- 5 Jablonska S, Orm G, Glinski G, *et al*. Morphology and immunology of human warts and familial warts. Fifth Munich Symposium on Microbiology. Bachmann P, ed. 1980:107-31.
- 6 Gross G, Pfister H, Hagedorn M, Gissman L. Correlation between human Papilloma virus (HPV) type and histology of warts. *J Invest Dermatol* 1982;78:160-4.
- 7 Jensen AB, Rosenthal JD, Olson C, *et al*. Immunologic relatedness of papilloma viruses from different species. *J National Cancer Institute* 1980;64:495-8.
- 8 Dyson JL, Walker PG, Singer A. Human papillomavirus infection of the uterine cervix, histologic appearance in 28 cases identified by immunohistochemical technique. *J Clin Pathol* 1984;37:126-30.
- 9 Kurman RJ, Shah KH, Lancaster WD, Jensen AB. Immunoperoxidase localization of papillomavirus antigens in cervical dysplasia and vulvar condylomas. *Am J Obstet Gynecol* 1981;140:931-5.
- 10 Sternberger LA. *Immunocytochemistry*. Englewood Cliffs, NJ, Practice Hall, 1974.
- 11 Gross G, Ikenberg H, Gissmann L, Hagedorn M. Papillomavirus infection of the anogenital region: Correlation between histology, clinical picture and virus type. Proposal of a new nomenclature. *J Invest Dermatol* 1985;85:147-52.
- 12 Sehgal VN, Koranne RV, Srivastava SB, Gupta MM, Luthra UK. Clinopathology and immunohistochemistry of genital warts. *Int J Dermatol* 1988;27:690-4.
- 13 Gimeno C, Vilata JJ, Sanchez JL, Lloret A, Fortea M. Bowenoid papulosis: Clinical and histological study of eight cases. *Gemtourin Med* 1987;63:109-13.
- 14 Guillet G, Brown L, Shah K, Ferenczy A. Papillomavirus in cervical condylomas with and without associated cervical intraepithelial neoplasia. *J Invest Dermatol* 1983;81:513-6.
- 15 Lack EE, Jensen AB, Smith HG, Heaby GB, Pass F, Vowter GF. Immunoperoxidase localization of human papillomavirus in laryngeal papilloma. *Intervirology* 1980;14:148-51.
- 16 Patel D, Shepherd PS, Naylor JA, McCance DJ. Reactivities of polyclonal and monoclonal antibodies raised to the major capsid protein of human papillomavirus type 16. *J Gen Virol* 1989;70:69-77.
- 17 McLean CS, Churcher MJ, Meinke J, *et al*. Production and characterisation of monoclonal antibody to human papilloma virus type 16 using recombinant vaccinia virus. *J Clin Pathol* 1990;43:488-92.

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